Size-dependent change in body shape and its possible ecological role in the Patagonian squid (*Doryteuthis gahi*) in the Southwest Atlantic

Jessica B. Jones [a, b, c*], Graham J. Pierce [a, d, e], Fran Saborido-Rey [e], Paul Brickle [a, c], Frithjof C. Kuepper [a], Zhanna N. Shcherbich [b], Alexander I. Arkhipkin [a, b]

[a] School of Biological Sciences, University of Aberdeen, Zoology Building, Tillydrone Avenue, Aberdeen, AB24 2TZ, Scotland, UK

[b] Falkland Islands Fisheries Department, Stanley, FIQQ 1ZZ, Falkland Islands

[c] South Atlantic Environmental Research Institute, Stanley Cottage, Stanley, FIQQ 1ZZ, Falkland Islands

[d] CESAM and Departamento de Biologia, Universidade de Aveiro, Av. Padre Fernão de Oliveira, 3810-193, Aveiro, Portugal

[e] Instituto de Investigacións Mariñas (CSIC), Eduardo Cabello 6, 36208, Vigo, Spain

[*] Corresponding Author: Number: +500 27260, Email: JJones@fisheries.gov.fk

ORCID number: 0000-0003-3098-6811 – Jones, 0000-0003-1273-7109 – Kuepper, 0000-0002-4744-4501 - Pierce

Abstract

Cephalopods are a versatile group with several mechanisms in place to ensure the success of future generations. The Patagonian long-finned squid (*Doryteuthis gahi*) populations on the southern Patagonian shelf are believed to be genetically homogenous but mechanisms connecting them geographically and temporally are unresolved. Individual growth is highly variable within cephalopod populations and is likely to affect individual patterns of migration and, thus, population connectivity as a whole. Therefore, this study aimed to make inferences about

population structure by analysing the size at which individuals were mature and aimed to describe the intrapopulation growth (allometric) trajectories of body shape, using landmark-based geometric morphometric techniques to describe phenotypes. Samples were collected from June 1999 to November 2017 around 52°S and 58°W. Smoothing curves from binomial generalised additive models (GAMs) suggested two size modes of maturity in females and one or multiple modes in males dependent on year and season. There was a gradual elongation of the mantle and an increase in the relative fin size throughout ontogeny. Shape scores from geometric morphometric shape coordinates revealed a continuous non-linear allometric trajectory with a significantly different slope angle for males exceeding 20.1 cm dorsal mantle length (DML). At the extreme of this continuum, the largest 'super-bull' form had a substantially more elongated body shape, a heavier fin and a larger fin area compared to the rest of the population, a body shape associated with enhanced swimming performance which may help to maintain population connectivity. The prevalence of these rare super-bulls in the fishery varied widely between years, suggestive of phenotypic plasticity. This study provides evidence that the *D. gahi* population on the southern Patagonian shelf has a complex population structure with high intraspecific variation.

1 Introduction

Populations of animals often incidentally assume risk-spreading strategies that reduce the potential for population collapse. In many cases, a multi-generational structure provides a reserve of several year-classes of reproductively viable adults and genetic variability that reduces this risk of collapse or extinction (O'Dor 1998). Conversely, most cephalopods are characterised by short, often annual life-cycles with a complete turnover in biomass every one to two years. They therefore do not have a multi-generational structure to buffer against fluctuations in spawning and recruitment success (Pierce and Guerra 1994; Boyle and Rodhouse 2008). The flexibility inherent within many cephalopod populations may allow them to persist within the heterogeneous marine environment without a multi-generational buffer in place (O'Dor 1998). Although this is a group selectionist argument for phenotypic plasticity, it is plausible if many populations exist and population persistence depends on plasticity.

Virtually every facet of cephalopod life-history examined to date has revealed an incredible capacity for life-history plasticity (Pecl and Jackson 2008). Loliginid squid often exhibit extended spawning, multiple peaks of spawning and pulses of recruitment within a single spawning season (Boyle and Boletzky 1996; Winter and

54 Arkhipkin 2015). Growth estimates often reveal high variability in size, with asynchronous growth and
55 maturation and substantial differences in growth-related parameters between spawning cohorts or seasonal
56 groups (Moreno et al. 2005). Within a single spawning season, multiple size modes of maturity are often found.
57 Male loliginid squid often have two clear size modes described (Guerra and Rocha 1994; Boyle et al. 1995;
58 Moreno et al. 2005; Hastie et al. 2009), but for *Loligo forbesii* evidence exists that there are three size modes
59 (Collins et al. 1999). Multiple size modes are not only apparent in male loliginid squid but have been found in
60 females of *L. forbesii* by Guerra and Rocha (1994), with similar findings implicit in the results of Collins et al.
61 (1999). This variability is thought to be largely a result of their response to external environmental cues,
62 particularly temperature (Forsythe 1993, 2004; Moreno et al. 2007). The complicated pattern of inshore-offshore
63 migration in loliginid squid, in addition to the extended spawning season mean that individuals of the same
64 population are exposed to very different environmental conditions, dependent on ontogenetic stage and hatching
65 date.

66 It is common in loliginid squid populations for mature males to exhibit two alternative forms of mate acquisition
67 on spawning grounds, with their behaviour dependent on body size (Hanlon and Messenger 1996). Large males,
68 referred to as 'bull' males have dominant behaviour forming mating pairs with females. Smaller subordinate
69 individuals or 'sneaker' males are of similar size or smaller than females. The small size of 'sneaker' males
70 facilitates a close approach to mating pairs where they then attempt to elicit extra-pair copulations (EPC's;
71 Hanlon and Messenger 1996). This type of mating system has been observed in the giant Australian cuttlefish
72 *Sepia apama* (Hall and Hanlon 2002), the chokka squid *Loligo reynaudii* (Hanlon et al. 2002), the oval squid
73 *Sepioteuthis lessoniana* (Wada et al. 2005) and the longfin inshore squid *Doryteuthis pealeii* (Shashar and
74 Hanlon 2013). The evolution of mating systems such as these is driven by mechanisms of sexual selection, with
75 the operational sex ratio on spawning grounds often biased towards males (Hall and Hanlon 2002; Olyott et al.
76 2006). This results in intrasexual competition between males for access to mates and, consequently, size
77 dimorphism (Alcock 2003). Multiple modes of mature males are often attributed to growth rates arising from
78 different environmental conditions, but have also been ascribed to alternative life history tactics and genetic
79 polymorphism (Hall and Hanlon 2002).

80 The Patagonian long-finned squid *Doryteuthis gahi* is a commercially important loliginid squid species
81 occurring in the Southwest Atlantic. In Falkland Islands waters it is a small squid typically attaining 13-17 cm
82 dorsal mantle length (DML), with a population structure consisting of two temporally distinct cohorts: the

spring spawning cohort (SSC) and the autumn spawning cohort (ASC) (Patterson 1988; Arkhipkin et al. 2008). These squid undertake ontogenetic migrations between their shallow inshore spawning grounds and their offshore feeding grounds on the outer shelf and shelf edge (Arkhipkin et al. 2004). Like most squid, they have a short lifespan of approximately one year (Hatfield 1991).

Growth within *D. gahi* has been shown to be highly variable (Arkhipkin and Roa-Ureta 2005), and the presence of different male size-groups (which were related to the sneaker/bull tactics) has been suggested but never investigated further (Arkhipkin and Middleton 2002). Unusually large *D. gahi* have been caught in the commercial fishery on a sporadic basis. These males are much larger and rarer than what could usually be considered a 'bull' male. Their ecological significance is unknown and it is not known whether these larger individuals represent a distinct behavioural morphotype or are a by-product of genetic or environmentally-driven phenotypic variation. A recent paper describing the anatomy and development of cephalopod tails suggested that a more streamlined body shape and a more centrally positioned fin should enhance the gliding abilities of squid and facilitate long, energy efficient migrations. This phenomenon is often documented in fish, with migrants with longer, steadier migrations being more streamlined and with longer caudal regions (Fraser and Bernatchez 2005; Crespel et al. 2017). The largest males of *D. gahi* were noted to have a more streamlined body shape than their smaller counterparts and were hypothesised to spatially connect subpopulations using their enhanced gliding abilities (Arkhipkin et al. 2015). The presence of anomalously large males such as this has been reported in other loliginid squid populations (Mangold 1987; Pierce et al. 2013).

The analysis of body shape can be used to distinguish between different species, sexes, groups or morphotypes. This is particularly important in the marine environment where there are often no obvious boundaries between populations. Traditionally, a series of angles and linear measurements between two anatomical points would be collected and analysed using multivariate statistics. The traditional morphometrics approach has limitations, such as the loss of information by simplifying the shape and the risk of selecting dimensions that do not adequately represent the actual shape variation (Adams et al. 2004; Braga et al. 2017). Over the past few decades, geometric morphometrics has been developed as an alternative method. This technique uses coordinates of biologically definable 'landmarks' or outlines to analyse shape and size and visualises 'deformations' of these morphometric points, therefore minimising this loss of information and retaining more detail about the geometry of the structure. Despite the advantages, landmark-based geometric morphometrics has rarely been undertaken for cephalopods and was initially applied to hard structures such as cuttlebone

(Neige and Boletzky 1997; Neige 2003), statoliths (Lombarte et al. 2006) or more recently beaks (Fang et al. 2017; Fang et al. 2018). Only in recent years has geometric morphometrics been applied to study squid body shapes (Crespi-Abril et al. 2010; Braga et al. 2017; Schroeder et al. 2017).

Previously, studies that have applied morphometric analyses to *D. gahi* have focused on geographic variation but have also revealed sexual dimorphism. The first electrophoretic and morphometric analysis was undertaken to study the genetic population structure of *D. gahi* samples from Falkland Islands waters (Carvalho and Pitcher 1989). Analysis of linear body measurements revealed morphologically distinct individuals from different geographical locations which were not distinct genetically. It was suggested that environmental differences in space and/or time were responsible for the observed morphological variation (i.e. phenotypic plasticity, Carvalho and Pitcher 1989). The second study used discriminant analysis to investigate the differences in statolith morphometric measurements between samples from Northern Peru and the Falkland Islands. There was significant sexual dimorphism in both regions and significant differences between regions for individuals of the same sex (Vega et al. 2001). The third study found significant differences in linear morphometric body measurements between specimens collected in Peru, Southern Chile and the Falkland Islands, as well as a greater sexual dimorphism in specimens collected from Falkland Islands waters compared to the other two geographic locations (Vega et al. 2002). There have been no morphometric studies on this species regarding the possible existence of different male forms (which may be linked to different reproductive strategies).

This study aims to examine aspects of flexibility in size and body shape within the Falkland Islands *D. gahi* population. Life cycle plasticity will be investigated by analysing size distribution and seasonal and inter-annual changes in the size-at-maturity. Multiple modes of mature individuals at different lengths may reveal the presence of different morphs with different patterns of growth. Seasonal and inter-annual changes in the modal size of mature individuals would be expected as each season and year represents a different cohort. However, the size at which these modes occur will aid in our understanding of the population structure of both sexes. The change in proportions related to variations in body size, i.e. ontogenetic allometry, will be investigated using geometric morphometrics to investigate the presence of distinct morphotypes, size groups and growth trajectories and any sexual dimorphism.

2 Material and Methods

2.1 Sample collection

140 Samples of *Doryteuthis gahi* were collected within the Falkland Islands Inner Conservation Zone (FICZ) from
141 June 1999 to November 2017 in a depth range of 60-306 m (Fig. 1). Samples were obtained by scientific
142 fisheries observers on commercial trawling vessels during the two fishing seasons (Season 1: March-May,
143 Season 2: July-October). Samples were also obtained during research cruises on the *RV Dorada* and *RV Castelo*
144 to provide samples during months outside the commercial fishing season. Data were collected to incorporate all
145 seasons, multiple years, a range of sizes and both sexes to obtain an overall impression of the population's body
146 shape variation. Due to their rarity, non-random samples of unusually large individuals >25 cm DML were
147 collected in 2014 and 2015 when there was a substantial influx of these individuals into the fishery (these were
148 combined with random samples to form the geometric morphometric dataset and were also incorporated into an
149 analysis of fin and mantle weight as described below). In doing so, the assumption of random sampling is
150 violated, requiring some changes to the strategy for geometric morphometric analysis (see below). All samples
151 were frozen on-board immediately for further analysis in the Falkland Islands Fisheries Department laboratory.
152 To prevent warping of morphological characteristics, which can happen with repeated freezing and thawing,
153 each sample was frozen as quickly as possible to prevent degradation and was defrosted only once at room
154 temperature prior to analysis (van der Vyver et al. 2016).

155 *2.2 Historical data*

156 Generalised linear models (GLMs) are often used to estimate the maturity ogive. However, since binomial
157 GLMs assume a linear response between the response and explanatory variables (after applying a logit
158 transformation), potential curvilinear relationships were explored by fitting binomial generalised additive
159 models (GAMs) to data from each sex separately to determine whether any modes in size-at-maturity could be
160 distinguished. GAM modelling was performed using the 'mgcv' package within the R environment (Wood
161 2011; R Core Team 2016). All records within a ten-year period (spanning from January 2007 to December
162 2017) of *D. gahi* entered into the Falkland Islands fisheries department database that had a DML (±0.5 cm, Fig.
163 2a), total weight (TW) and a maturity stage were used in the GAMs (males = 44,439, females = 46,296). The
164 binomial response variable maturity was estimated in all specimens based on gonad developmental stages
165 following Lipinski (1979): stages 1 to 4 representing immature individuals (0) and stage 5 representing mature
166 specimens (1). Prior to analysis, data were graphically inspected following procedures described in Zuur et al.
167 (2010). Apparently erroneous values (from visual inspection) were cross-referenced against the original data
168 sheets and either corrected or deleted. As size-at-maturity has been found to be highly variable in other loliginid

169 species, and dependent on location and time of year (Augustyn et al. 1992), explanatory variables were included
170 to explore any changes in the proportion of mature individuals of each size (DML) over time and space. Year of
171 collection (nominal with 10 levels) was explored as an explanatory variable and the interaction between size
172 (smoother) and year was explored using the 'by' command in the 'mgcv' package. It applies a size smoother on
173 the data for each year separately. Models with and without the inclusion of separate smoothers for each year
174 were compared using a chi-squared test. Models with the inclusion of separate size smoothers for the nominal
175 variable fishing season (defined as commercial Season 1: March-May and Season 2: July-October) were also
176 explored. The addition of longitude and latitude as a smooth term was investigated. Optimal GAMs were
177 selected via forwards selection on the basis of the lowest value of Akaike's Information Criterion (AIC) and
178 Akaike weights (Wagenmakers and Farrell), providing that there were no serious patterns in residuals and all
179 remaining explanatory variables had a significant effect.

180 All records of fully mature male and female *D. gahi* were used to determine size distributions of mature
181 individuals for both sexes. All mature males were categorised by year and fishing season. The percentage
182 contribution of unusually large males (set at 25 cm DML based on examination of the size distributions) to the
183 total number of mature males caught in each year was calculated by fishing season.

184 A sub-sample of 1,701 individuals (males = 1,212 and females = 489) had fin weight and mantle weight
185 collected in addition to TW (all ± 1 g). Two morphometric indices were calculated to determine the allometric
186 change in fin weight (FWTI) and mantle weight (MWTI) with DML, defined as:

187 $$WI = log(W)/log(DML)$$

188 Where *W* is either fin weight (FWT) or mantle weight (MWT), and *WI* are the corresponding indices. Indices
189 were calculated and plotted against DML.

190 *2.3 Geometric morphometrics*

191 *2.3.1 Imaging and landmarking*

192 Males (n = 159) and females (n = 71) were selected to cover the whole size range and were photographed for
193 geometric morphometrics (Table 1). All specimens were caught within the FICZ, with only two samples taken
194 outside the 'loligo box', the designated area for commercial fishing (Fig. 1). Undamaged specimens in a good
195 state of preservation were chosen for analysis. Photographs were taken on defrosted specimens with a Sony

196 Digital Camera (23 megapixels), ensuring the focal plane of the camera and the frontal plane of the specimen
197 were parallel and a scale was included to standardise size. Specimens were positioned with the ventral surface
198 up to prevent fins from hanging. Six 2D landmark coordinates were obtained using TPSDig 2 v.2.26 (Rohlf
199 2015) (Fig. 2b). Of these, three were type I (location of point determined by obvious biologically homogenous
200 structure), one was type II (location defined by obvious geometry – the point of greatest curvature on the fin)
201 and two were type III (points defined by another point – midpoint of the mantle aligned with type I points)
202 (Bookstein 1992). Landmarks were selected assuming symmetry in the sagittal plane to reduce degrees of
203 freedom during analysis (Crespi-Abril et al. 2010).

204 An additional type III landmark was digitised to un-bend specimens (Fig. 2b), with four points required to fit the
205 quadratic curve that adjusts for curvature (the additional landmark and landmarks 4, 5 and 6). However, the R^2
206 values for the quadratic curves fitted in tpsUtil (Rohlf 2015) were particularly low, and a comparison of
207 statistical analyses using raw and unbent data indicated that unbending had little effect on the statistical output.
208 Thus, raw landmarks with no unbending were finally used during analysis.

209 *2.3.2 Size*

210 Further analyses of landmark coordinates were undertaken in the package 'geomorph' in the R environment
211 (Adams and Otárola-Castillo 2013). Centroid size (CS), a measure of geometric scale, was calculated as the
212 square root of the sum of the squared deviations of landmarks from a centroid (Bookstein 1992) and was used as
213 a proxy for body size. In the present study, Procrustes analysis of variance (ANOVA) indicated that CS was
214 highly correlated with DML ($R^2 = 0.97$, $p = 0.001$).

215 *2.3.3 Shape*

216 To remove non-shape variation, landmark configurations of each specimen were submitted to Generalised
217 Procrustes Analysis (GPA) to superimpose each specimen onto a common coordinate system. Landmark
218 configurations were translated so their centroid was at the origin of the coordinate system, then were scaled (to
219 unit CS) and rotated. This was an iterative process with all landmark configurations rotated around their centroid
220 repeatedly to minimise the squared distance between corresponding landmarks. Once all the landmarks were
221 aligned, the mean landmark configuration was computed, from here onwards referred to as the consensus shape.
222 The GPA-aligned coordinates are the Procrustes coordinates, and were used as the shape variables in further
223 statistical analyses.

224 *2.3.4 Growth Trajectories*

225 Growth trajectory analysis was undertaken following the methods described in Rodríguez-Mendoza et al.
226 (2011). A multivariate regression was used to determine growth trajectories, characterising morphological
227 changes of the Procrustes coordinates (shape) in response to the log-transformed CS. The 'procD.lm' function in
228 R was used to perform a Procrustes ANOVA with permutation procedures (set to 10,000 iterations) to test the
229 null hypothesis of independence between shape and size. To visualise the strength of association between size
230 and shape, shape scores were calculated according to Drake and Klingenberg (2008) and plotted against
231 log(CS). The variation in growth trajectories by sex, year of collection, season and maturity stage were
232 investigated by plotting shape scores against log(CS) by each variable. For visual purposes, maturity was pooled
233 into immature (stages 1-2), maturing (stages 3-4) and mature (stage 5). To show the shape changes associated
234 with growth, deformation grids were visualised using a thin-plate spline (TPS) approach (Bookstein 1992) to
235 display deviation from the consensus shape, magnified x2 to emphasise differences. The relative amount of
236 mantle body shape variation attributable to sex, year of collection and the interactions between size and these
237 co-variables were explored using a multivariate analysis of variance (MANOVA) on geometric shape
238 coordinates.

239 As the male allometry appeared to be non-linear, similarity of growth trajectories of male squid from different
240 size groups (4 groups; 6.5-10 cm, 10.1-20 cm, 20.1-30 cm and 30.1+ cm) were evaluated, following the
241 approach as described in Zelditch et al. (2003). Regression vectors were obtained describing the ontogeny of
242 shape over each size group. Components of the vector are regression coefficients of the shape scores on log(CS).
243 To compare size-groups the angle between regression vectors was calculated pairwise, then the between-group
244 angle was compared with the upper 95% confidence interval of the within-group angle ranges (assessed by
245 bootstrapping using 10,000 runs). If the between-group angle exceeded the 95% confidence interval of the two
246 within-group angles, the difference was judged statistically significant at the 5% level. To investigate the
247 influence of the addition of non-random samples to the geometric morphometric dataset, an additional
248 multivariate regression was undertaken (non-random samples removed), with Procrustes coordinates as the
249 response variable and sex and log(CS) as putative co-variables.

250

251

3 Results

3.1 Historical Data

The candidate models which explored the proportion of mature individuals of *Doryteuthis gahi* of each size using binomial GAMs can be found in Table 2. In the simplest model (MN1), the relationship between size and maturity was non-linear for both sexes (edf = 4.44 for males and 5.67 for females, for the smooth of DML), but multiple modes of maturity were not clearly discernible when year was not included as a variable in the male data (Appendix 1). Conversely, females showed evidence of two different modes of maturity in MN1. The inclusion of separate smooths for each year lowered the AIC and significantly improved model fit for both males (MN1 vs MN3, $\chi^2 = 2777.3$, $p < 0.001$) and females (MN1 vs MN3, $\chi^2 = 881.5$, $p < 0.001$). There was significant inter-annual variability in the size-maturity relationship for both sexes (Fig. 3) which was most pronounced for females (Fig. 3a). For males (Fig. 3b), the size-maturity relationship followed a similar pattern for most years, but the size at which individuals matured showed some inter-annual variation. Out of the entire ten-year period, there is evidence of two clear modes in size-at-maturity in 2016 for males (edf = 6, the highest edf of the ten-year period indicating a more complex pattern), with a second mode commencing from 28 cm DML onwards. Three years within the ten-year period had complex patterns with multiple modes and the majority of years (six) had simple (but still non-linear) patterns with no modes discernible (MN3, Fig. 3b). Inter-annual patterns in the size-maturity relationship were more complex and variable in females (MN3, Fig. 3a).

Predictors retained in the most parsimonious model (MN7 for both sexes, which both had high Akaike weights of 0.999) of maturity included separate smooth terms for each fishing season for the effect of DML, a smoothing term for location, and year included as a factor (all of which were highly significant). This model explained 55% of the deviance in females and 44.8% in males. In relation to the smoothing terms applied to each season for the effect of DML (MN7, Fig. 4), the smooth for males collected during the first season had the lowest edf (edf = 3.28, Fig. 4c) indicating a less complex trend, closer to linear. Males in the second season had the most complex trend with evidence of multiple size-modes, the clearest of which are evident at 24 and 31 cm (edf = 8.78, Fig. 4d). In both sexes, smoothing curves became more complex in the second season. For females, two modes in maturity were evident in both seasons, though this trend was more pronounced in the second season (Fig. 4b). Partial effects of the smooth using longitude and latitude suggested that the most mature males were located to the west of the FICZ and in shallow coastal waters around the Falkland Islands and that most mature females were located in shallow coastal waters (refer to Appendix 2).

281 Mature (maturity stage 5) male *D. gahi* ranged from 5 to 44 cm DML (Fig. 5a), a broader range than was seen in
282 mature females (7-29 cm DML, Fig. 5b). Length frequency distributions were skewed to the right. Mature
283 animals of both sexes had a similar modal size (males – 14 cm DML, females – 15 cm DML), with most mature
284 individuals falling in the range of 13-17 cm DML. However, mean size of mature males was larger than that of
285 females due to a substantially larger tail in size distribution i.e. in males there were individuals at the extreme
286 positive end of the size distribution. Only 35 females were recorded that exceeded 25 cm DML in 29 years of
287 sampling, 0.04% of the mature female population. Mature males larger than 25 cm DML were also a rare
288 component of the fisheries catch (Fig. 5b), but were much more frequent than large females, constituting 1.17%
289 of the entire mature male catch. Most large males entered the fishery within the second season, in the latter half
290 of the year (except for 2012 when a large number of these individuals were caught in a research cruise in April,
291 Fig. 6). Their prevalence in the fishery varied widely between years, with peak catches of large males in 1993
292 (5.78% of mature males), 2003 (2.40%) and 2012 (5.38%).

293 Indices of fin weight (FWTI) and mantle weight (MWTI) are plotted against DML in Fig. 7. Both indices
294 increased with DML and appeared to reach an asymptote in males at approximately 35-40 cm DML (Fig. 7).
295 These trends indicate that the weight of both fin and mantle increased relative to mantle length (and
296 correspondingly with squid size), becoming more muscular (and powerful) in larger animals of up to 35-40 cm
297 DML.

298 *3.2 Geometric Morphometrics*

299 The multivariate regression of shape (Procrustes coordinates) on log(CS) was highly significant, demonstrating
300 substantial ontogenetic allometry ($Z = 4.82$, $p < 0.001$). Shape scores plotted against log(CS) showed an almost
301 linear increase in with size for males and females (Fig. 8), but a shift in the shape-growth pattern for males
302 exceeding a log(CS) of ~2.5 (Fig. 8a). Females (Fig. 8b) grew to a log(CS) of ~2.5, which is approximately
303 equivalent to 15 cm DML (given the highly significant relationship between CS and DML) and is within the
304 cited average adult size of this species (13-17 cm DML, Arkhipkin et al. 2013). In males, size extended beyond
305 the normal adult size range due to the inclusion of large non-random individuals (Fig. 8a). The variation in
306 shape scores is similar between females and males with a log(CS) <2.5. Given the patterns in maturity and the
307 shift in the shape-growth pattern it is possible that two-size dependent allometries are present in the male
308 population, given that there are immature individuals within the mean adult size range and maturing individuals
309 exceeding this size range. Shape for larger individuals appears to change in a different way to their smaller

310 counterparts. A larger sample size, particularly of mature males within the mean adult size range, would be
311 required to confirm this, given the high amount of variation in shape scores for squid of the same size. When
312 labelled by year and season there is no evidence to suggest that seasonal or inter-annual variation are driving
313 these patterns (Appendix 3). Examination of Thin Plate Spline (TPS) deformation grids of the most extreme
314 shape scores indicate that individuals with the smallest shape scores are characterised by a shorter fin and wider
315 mantle, whilst those with the largest shape scores had a narrower mantle and longer fins (Fig. 8).

316 Deformation grids (showing the mean shape of each size-group relative to the consensus shape) confirm this
317 trend, when separated by sex and into 10 cm size groupings (Fig. 9) and show that differences in shape with
318 size, for males in particular, are striking and continuous. Males and females in the 6.5-10 cm DML size
319 groupings both have similar shapes, with a smaller fin relative to the consensus configuration (Fig. 9a/b). A
320 similar shape is also found for males and females in the 10.1-20 cm DML size-group, which closely resemble
321 the consensus shape – this size-group of mature individuals are most common within the population. Only males
322 are found in the last two largest size groups, showing an extreme increase in the relative size of the fin, a larger
323 fin area and an elongation of the body shape. Allometric trajectories were compared pairwise by calculating the
324 angle between the regression vectors of each size group (Table 4). The allometric patterns of the two smallest
325 size groups (6.5-10 and 10.1-20 cm DML) differ significantly to the larger size groups (20.1-30 and 30+ cm
326 DML) for all pairwise combinations, suggestive of a shift in growth (which could be maturation). The largest
327 angle (79°) was found between individuals from 10.1-20 cm (the usual size range for mature males but also
328 including immature individuals) and those of 30.1 cm + DML.

329 The multivariate regression of shape (Procrustes coordinates) on log-transformed CS, sex and year of collection
330 indicated highly significant effects of all explanatory variables (permutation test with 10,000 random
331 permutations, $p < 0.001$, Table 3), with the effect of size explaining 72.6% of the shape variation. The inclusion
332 of an interaction between log(CS) and sex significantly improved model fit, with individuals of each sex having
333 a significantly different slope ($F = 8.99$, $p = 0.002$). All interactions, including the three-way interaction, were
334 highly significant except the interaction between sex and year of collection ($p = 0.87$). The Z-value, the
335 preferred measure of effect strength, was largest for size ($Z = 6.56$), followed by the interaction between size
336 and year ($Z = 6.48$). Multi-variate analysis without the addition of non-random samples confirmed that log(CS)
337 and sex had a significant effect on shape (Appendix 4).

338

4 Discussion

In the present study we found the size-range of mature male individuals was much broader than found in mature females and males obtained a much greater maximum size, which is typical in other loliginids (Coelho et al. 1994; Collins et al. 1995; Raya et al. 1999). Like *Doryteuthis sanpaulensis* from the coastal waters of north-eastern Patagonia (Barón and Ré 2002), male *D. gahi* had a single mode in size-at-maturity for 6 out of 10 investigated years, the exceptions being 2016 when two clear size modes were observed with the second, larger, mode exceeding 28 cm DML and three years where the pattern indicated more than two modes of maturity. Examining size-at-maturity separately for the two commercial fishing seasons revealed additional complexity. The smoother for the effect of DML on maturity in the first season was almost linear for males, indicative of one size-at-maturity. However, patterns of maturity were more complex in the second season for both sexes, with males showing two or more modes at the extremes of the size range when unusually large males most often appear in the fishery. The one size-at-maturity results found in most years and the first season are contrary to previous studies on other loliginid species, which found two clear modes of maturity in males of; *Loligo vulgaris* (Coelho et al. 1994; Guerra and Rocha 1994; Raya et al. 1999; Moreno et al. 2005), *Loligo forbesii* (Guerra and Rocha 1994; Boyle et al. 1995; Rocha and Guerra 1999; Collins et al. 1995) and *Alloteuthis subulata* (Hastie et al. 2009). The multiple modes that occur sporadically on an inter-annual basis and during the second season in males are particularly interesting. What is surprising is that for this species, females had two sizes-at-maturity for both seasons and a much more complex inter-annual pattern with evidence of multiple modes in most years. Multiple modes in females have been observed in *L. forbesii* off the west coast of Scotland, where at least two sizes-at-maturity were identified in both sexes (Guerra and Rocha 1994; Boyle et al. 1995; Collins et al. 1999).

There are several potential explanations for the phenomenon of multiple modes in maturity (which are not mutually exclusive), with multiple modes potentially arising from one, or a combination of the following (summarised in Guerra and Rocha 1994 and Boyle et al. 1995);

1. The existence of short and long reproductive cycles resulting from different rates of maturity (Mesnil 1977; Coelho et al. 1994; Boyle et al. 1995). Squid hatched from eggs early in the breeding season mature rapidly and mate with females from their own generation/cohort (annual lifecycle). Environmental factors inhibit and delay the maturation of those hatching later in the breeding season.

368 This results in a larger size and extended lifecycle, with these individuals breeding with the following
369 generation/cohort.
370 2. Individuals of the same age having different feeding, migration or distribution patterns, leading to
371 different growth rates. Different rates of growth may result in a 'sneaker/bull' breeding strategy, as
372 these are size-dependent behavioural forms (Moreno et al. 2005).
373 3. The mixing of populations from different sources with different size characteristics (Boyle et al. 1995).
374 4. Multiple pulses of recruitment with squid of a different size recruiting into the fishery, resulting in a
375 different size due to the different environmental conditions experienced by each successive pulse and a
376 more complex pattern of maturity (more than two modes). Multiple pulses of recruitment are
377 considered as a factor during the management of this particular stock (Winter and Arkhipkin 2015).

378 Given that two or more modes were observed in 4 out of the 10 years studied, it is likely that these different
379 size-groups of squid arise from the specific environmental conditions within certain years (i.e. phenotypic
380 variation). It is often suggested that the existence of different groups of squid developing under different
381 environmental conditions, especially under different water temperatures, is likely to result in different growth
382 strategies (Moreno et al. 2005, Vila et al. 2010). GAM analysis in the present study revealed inter-annual,
383 seasonal and spatial variation in the relationship between the proportion of mature individuals and length for
384 both sexes, which implies that environmental conditions have a significant influence. It is not clear at this point
385 whether environmental variability results in delayed maturation and an extended lifecycle of the larger size
386 modes in either sex, or if they are the same age and the larger mature animals simply have accelerated growth.
387 However, previous ageing studies have found no evidence that this species life cycle extends much beyond one
388 year (Hatfield 2000).

389 The lack of multiple size modes (in most years) could be a result of sampling, as the majority of squid were
390 sampled on their offshore feeding grounds during a narrow 'window' in time (the fixed duration of fishing
391 seasons) and space (depths of predominantly 140-200 m) within which the fishery operates (Hatfield and
392 Murray 1999), and therefore includes growing (but already mature) males. Direct field observations in spawning
393 grounds would be required to corroborate our results, but unlike other loliginid species which form dense, easy
394 to locate spawning aggregations, spawning locations for *D. gahi* are more ambiguous. *D. gahi* is known to
395 choose exposed sites on the outer edge of kelp forests, attaching their eggs to kelp stipes at shallow depths (<20
396 m), but the density of egg masses found during previous surveys was low when compared to the adult biomass

(Arkhipkin et al. 2000). The complete distribution of spawning sites is yet to be fully resolved, with an egg mass recovered at depths of 69-71 m indicating that spawning may occur much deeper than previously believed (Laptikhovsky 2008).

Generally, this study found that small immature individuals were characterised by smaller, shorter fins and a wider mantle, whilst the largest mature animals had relatively narrower and more elongate mantles and larger fins that extended along a larger portion of the mantle, most evident in the deformation grids of the maximum and minimum shape scores in Fig. 8. FWTI and MWTI showed that the fins and mantle became more muscular (and powerful) with increasing DML. Most squid, including *D. gahi,* hatch with small rudimentary fins and a saccular body (Hoar et al. 1994; Bartol et al. 2008; Arkhipkin et al. 2013). There is a gradual change in shape throughout ontogeny as the body becomes more streamlined to minimise drag and the fins become more prominent which likely optimise swimming capabilities. In a study synthesising intraspecific patterns in the allometric coefficient (*b*) of the length-weight regression across a variety of pelagic animals, cephalopods (along with salps, which also locomote by jetting) were suggested to radically elongate their body shape as they enlarged (Hirst 2012). Males displayed a significant ontogenetic elongation and were more elongate than females when mature (this elongation is evident in the thin plate spline deformation grids and Fig. 2).

There is a continuous elongation and increase in fin area not only with maturation but also with size once maturity is achieved. Deformation grids indicating the change in mean shape between size groups indicated that males exceeding 30.1 cm had a more elongate shape and more prominent fins that those of 20.1 to 30 cm (Fig. 9e/f). Pairwise comparisons of the angle between the regression vectors of these two size-groups found a significant angle of 76.7°. The streamlined mantle shape and developed, more centrally positioned fins of the largest male individuals are likely to offer less resistance to fluid flow and enhance their gliding capabilities, and so facilitate long energy-efficient migrations that would not require a substantial contribution of the mantle muscles for jetting (Arkhipkin et al. 2015). Shape scores and pairwise analyses of angles between regression vectors of different size groups suggest that two different allometric trajectories are apparent, which given the sporadic appearance of the larger morph is linked to environmentally-driven differences in growth. Adult squid of this species often achieve sizes of 13-17 cm DML, with a log(CS) of approximately 2.5 equating to 15 cm DML. With immature and maturing individuals found above this size, it is likely that larger individuals have a different morphology to the main population. Though further sampling is required to establish exactly where each trajectory starts and ends (as there is overlap) most individuals exceeding 20.1 cm DML are likely to be

426 part of this second growth trajectory, given the significantly different slope angle for males exceeding this size.
427 There was a significant inter-annual effect of year on shape as well as significant interactions between size and
428 year, and the three-way interaction between size, sex and year. Thus, it is likely that the difference in size and
429 shape is somehow attributable to phenotypic plasticity.

430 Individuals exceeding 20-25 cm DML rarely occur in the fishery, have a significantly different regression vector
431 angle (and therefore a different growth pattern) from males within the normal size range (10.1-20 cm DML),
432 and are an extreme body shape (sudden fin enlargement and elongation is visually evident in the deformation
433 grids of males exceeding 20.1 cm DML and is not visually evident prior to this), suggesting that they are
434 different to the rest of the population. The presence of morphs at extremes of the size continuum, with different
435 patterns of growth to the rest of the population, has been demonstrated in the Ommastrephid squid *Notodarus*
436 *gouldi* in Australia and Tasmania (Jackson et al. 2003). This study investigated age and growth on a broad
437 geographic scale and established that squid from Ulladulla were characterised by extreme small size, the
438 smallest gonads and a short lifespan. The authors suggested that environmental conditions were pushing *N.*
439 *gouldi* to its biological and physiological limits. This small morph was localised to one region, but flexibility in
440 the population dynamics allowed 'Ulladulla-like morphs' to occasionally appear at other locations, should the
441 appropriate conditions prevail (Jackson et al. 2003). The flexibility of *D. gahi* and the oceanographic conditions
442 in the regions are likewise considered to drive the appearance of large 'super-bull' morphs on the southern
443 Patagonian shelf.

444 Taking into account their extreme streamlined shape and large fins, the 'super-bulls' in the *D. gahi* population
445 are likely to have an advantageous shape that allows them to undertake longer energy-efficient migrations than
446 the rest of the population. This may help maintain connectivity between geographically discrete areas of the
447 population (Arkhipkin et al. 2015). Though they are a rare component of the catch, only low levels of gene flow
448 are required to prevent genetic divergence with only a few individuals required to maintain homogeneity
449 (Carvalho and Pitcher 1989). Tagging experiments on *L. reynaudii* showed that squid (particularly males)
450 moved between spawning grounds up to 100 km away, maintaining gene flow (Sauer 1995). Further work to
451 obtain the genetic identity of these super-bulls and the rest of the population by determining the entire
452 mitochondrial genome sequence would provide critical information on connectivity this species, as recently
453 demonstrated for other loliginid squid (Jiang et al. 2018).

454 Given that geographically and temporally separated individuals were not found to be genetically distinct in the
455 first morphometric and electrophoretic study on *D. gahi*, morphometric differences in space and time were
456 attributed to environmental heterogeneity (Carvalho and Pitcher 1989). Similarly, analysis of four microsatellite
457 loci from genomic DNA of *L. reynaudii* on the south and west coast of South Africa similarly found no
458 significant genetic structuring, despite morphometric analysis of soft body parts resulting in good discrimination
459 between different geographic regions. This geographic variation was suggested to be related to phenotypic
460 responses derived from regional environmental conditions rather than restriction of gene flow (van Der Vyver et
461 al. 2016). Environmental variables, particularly temperature, are often cited to have significant effects on the
462 growth and development of cephalopods, especially during early stages of ontogeny (Forsythe 1993, Rocha and
463 Guerra 1999; Hatfield 2000; Forsythe et al. 2001; Forsythe 2004). The Forsythe hypothesis states that increased
464 temperature during a squid's early growth phase may result in accelerated growth and potentially a larger
465 maximum size (Forsythe 1993, 2004). *D. gahi* hatched in austral summer (at higher temperatures) had a
466 significantly higher size-at-age than squid of the same age hatched in the winter months (Hatfield 2000). The
467 fact that the majority of the large super-bulls apparently recruited to the fishery within the second season and
468 that their presence in the fishery varied widely between years suggests that their occurrence may be (at least
469 partly) driven by extrinsic factors such as temperature. Other extrinsic factors such as food availability and
470 upwelling may also have a marked effect on squid size; winter-spring hatched individuals within the *L. vulgaris*
471 population in Galician waters were found to have a larger size-at-age than the summer-autumn hatched
472 individuals. It was hypothesised that the coastal summer-autumn upwelling, which noticeably increases
473 productivity in the area, coincides with their early stages of development resulting in high growth rates, with this
474 species closely associated with coastal waters. Conversely, winter-spring hatched individuals from the more
475 oceanic *L. forbesii* were smaller than their summer-autumn counterparts. It was hypothesised that their offshore
476 displacements mean that they do not benefit from the coastal upwelling, resulting in low growth rates (Rocha
477 and Guerra 1999).

478 In conclusion, geometric morphometrics appears to be a robust method of describing allometric body shape
479 variation in squid and is useful for visualising body shape change with deformation grids. Though this study has
480 furthered our understanding of the population structure of this species, future work into the environmental
481 conditions associated with the influx of these 'super-bull' males into the fishery and a whole genome genetic
482 evaluation of the population would be useful future steps to better understand the population dynamics of this
483 important commercial species.

Compliance with ethical standards

Conflict of interest

The authors have no conflict of interest to declare.

Research involving human participants and/or animals

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

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References

Adams DC, Otárola-Castillo E (2013) Geomorph: An R package for the collection and analysis of geometric morphometric shape data. Methods Ecol Evol 4(4): 393-399

Adams DC, Rohlf FJ, Slice DE (2004) Geometric morphometrics: Ten years of progress following the 'revolution'. Ital J Zool 71(1): 5-16. https://doi.org/10.1080/11250000409356545

508 Alcock J (2003) Animal Behavior: An Evolutionary Approach (7th edition). Sunderland: Sinauer Associates,
509 560 pp.

510 Arkhipkin AI, Middleton DA (2002) Sexual segregation in ontogenetic migrations by the squid *Loligo gahi*
511 around the Falkland Islands. Bull Mar Sci 71(1): 109-127

512 Arkhipkin AI, Roa-Ureta R (2005) Identification of ontogenetic growth models for squid. Mar Freshwater Res
513 56(4): 371-386. https://doi.org/10.1071/mf04274

514 Arkhipkin AI, Grzebielec R, Sirota AM, Remeslo AV, Polishchuk IA, Middleton DA (2004) The influence of
515 seasonal environmental changes on ontogenetic migrations of the squid *Loligo gahi* on the Falkland shelf.
516 Fish Oceanogr 13(1): 1-9. https://doi.org/10.1046/j.1365-2419.2003.00269.x

517 Arkhipkin AI, Laptikhovsky VV, Middleton DAJ (2000) Adaptations for cold water spawning in loliginid
518 squid: *Loligo gahi* in Falkland waters. J Molluscan Stud 66(4): 551-564.
519 https://doi.org/10.1093/mollus/66.4.551

520 Arkhipkin AI, Middleton DA, Barton J (2008) Management and conservation of a short-lived fishery resource:
521 *Loligo gahi* around the Falkland Islands. In: Am Fish Soc Symp 49(2): 1243

522 Arkhipkin AI, Hatfield EM, Rodhouse PG (2013) *Doryteuthis gahi*, Patagonian long-finned squid. In: Rosa R,
523 O'Dor R, Pierce GJ (ed) Advances in squid biology, ecology and fisheries. Part I-myopsid squids. Nova
524 Science Publishers Inc, pp 123-158

525 Arkhipkin AI, Weis R, Mariotti N, Shcherbich Z (2015) 'Tailed' cephalopods. J Molluscan Stud: 81(3): 345-
526 355. https://doi.org/10.1093/mollus/eyu094

527 Augustyn CJ, Llpiński MR, Sauer WHH (1992) Can the Loligo squid fishery be managed effectively? A
528 synthesis of research on *Loligo vulgaris reynaudii*. S Afr J Marine Sci 12(1): 903-918

529 Barón PJ, Ré ME (2002) Reproductive cycle and population structure of *Loligo sanpaulensis* of the northeastern
530 coast of Patagonia. Bull Mar Sci 71(1): 175-186

Bartol IK, Krueger PS, Thompson JT, Stewart WJ (2008) Swimming dynamics and propulsive efficiency of squids throughout ontogeny. Integr Comp Biol 48(6): 720-733. https://doi.org/10.1093/icb/icn043

Bookstein FL (1992) Morphometric tools for landmark data: geometry and biology. Cambridge University Press. https://doi.org/10.1017/cbo9780511573064

Boyle PR, Boletzky SV (1996) Cephalopod populations: definition and dynamics. Phil Trans R Soc Lond B 351(1343): 985-1002. https://doi.org/10.1098/rstb.1996.0089

Boyle P, Rodhouse P (2008) Cephalopods: ecology and fisheries. John Wiley & Sons. https://doi.org/10.1002/9780470995310

Boyle PR, Pierce GJ, Hastie LC (1995) Flexible reproductive strategies in the squid *Loligo forbesi*. Mar Biol 121(3): 501-508. https://doi.org/10.1007/bf00349459

Braga R, Crespi-Abril AC, Van der Molen S, Bainy MCRS, Ortiz N (2017) Analysis of the morphological variation of *Doryteuthis sanpaulensis* (Cephalopoda: Loliginidae) in Argentinian and Brazilian coastal waters using geometric morphometrics techniques. Mar Biodivers 47(3): 755-762. https://doi.org/10.1007/s12526-017-0661-z

Carvalho GR, Pitcher TJ (1989) Biochemical genetic studies on the Patagonian squid *Loligo gahi* D'Orbigny. II. Population structure in Falkland waters using isozymes, morphometrics and life history data. J Exp Mar Biol Ecol 126(3): 243-258. https://doi.org/10.1016/0022-0981(89)90190-1

Coelho ML, Quintela J, Bettencourt V, Olavo G, Villa H (1994) Population structure, maturation patterns and fecundity of the squid *Loligo vulgaris* from southern Portugal. Fish Res 21(1-2): 87-102. https://doi.org/10.1016/0165-7836(94)90097-3

Collins MA, Boyle PR, Pierce GJ, Key LN, Hughes SE, Murphy J (1999) Resolution of multiple cohorts in the *Loligo forbesi* population from the west of Scotland. ICES J Mar Sci 56(4): 500-509. https://doi.org/10.1006/jmsc.1999.0485

554 Collins MA, Burnell GM, Rodhouse PG (1995) Reproductive strategies of male and female *Loligo forbesi*
555 (Cephalopoda: Loliginidae). J Mar Biol Assoc U K 75(3): 621-634.
556 https://doi.org/10.1017/s0025315400039059

557 Crespel A, Dupont-Prinet A, Bernatchez L, Claireaux G, Tremblay R, Audet C (2017) Divergence in
558 physiological factors affecting swimming performance between anadromous and resident populations of
559 brook charr *Salvelinus fontinalis*. J Fish Biol 90(5): 2170-2193. https://doi.org/10.1111/jfb.13300

560 Crespi-Abril AC, Morsan EM, Barón PJ (2010) Analysis of the ontogenetic variation in body and beak shape of
561 the *Illex argentinus* inner shelf spawning groups by geometric morphometrics. J Mar Biol Assoc U K
562 90(3): 547-553. https://doi.org/10.1017/s0025315409990567

563 Drake AG, Klingenberg CP (2008) The pace of morphological change: historical transformation of skull shape
564 in St Bernard dogs. Proc R Soc Lond B Biol Sci 275(1630): 71-76. https://doi.org/10.1098/rspb.2007.1169

565 Fang Z, Chen X, Su H, Thompson K, Chen Y (2017). Evaluation of stock variation and sexual dimorphism of
566 beak shape of neon flying squid, *Ommastrephes bartramii*, based on geometric morphometrics.
567 Hydrobiologia 784(1): 367-380. https://doi.org/10.1007/s10750-016-2898-0

568 Fang Z, Fan J, Chen X, Chen Y (2018) Beak identification of four dominant octopus species in the East China
569 Sea based on traditional measurements and geometric morphometrics. Fish Sci 84(6): 975-985.
570 https://doi.org/10.1007/s12562-018-1235-0

571 Forsythe JW (1993) A working hypothesis of how seasonal temperature change may impact the field growth of
572 young cephalopods. In: Okutani A, O'Dor RK, Kubodera T (ed) Recent Advances in Cephalopod
573 Fisheries Biology. Tokai University Press, Tokyo, pp 133-143

574 Forsythe JW (2004) Accounting for the effect of temperature on squid growth in nature: from hypothesis to
575 practice. Mar Freshwater Res 55(4): 331-339. https://doi.org/10.1071/mf03146

576 Forsythe JW, Walsh LS, Turk PE, Lee PG (2001) Impact of temperature on juvenile growth and age at first egg-
577 laying of the Pacific reef squid *Sepioteuthis lessoniana* reared in captivity. Mar Biol 138(1) 103-112.
578 https://doi.org/10.1007/s002270000450

579 Fraser DJ, Bernatchez L (2005) Adaptive migratory divergence among sympatric brok charr populations.
580 Evolution 59(3): 611-624. https://doi.org/10.1554/04-346

581 Guerra A, Rocha F (1994) The life history of *Loligo vulgaris* and *Loligo forbesi* (Cephalopoda: Loliginidae) in
582 Galician waters (NW Spain). Fish Res 21(1-2): 43-69. https://doi.org/10.1016/0165-7836(94)90095-7

583 Hall KC, Hanlon RT (2002) Principal features of the mating system of a large spawning aggregation of the giant
584 Australian cuttlefish *Sepia apama* (Mollusca: Cephalopoda). Mar Biol 140(3): 533-545.
585 https://doi.org/10.1007/s00227-001-0718-0

586 Hanlon RT, Messenger JB (1996) Cephalopod behaviour. Cambridge University Press.
587 https://doi.org/10.1017/s0025315400041060

588 Hanlon RT, Smale MJ, Sauer WH (2002) The mating system of the squid *Loligo vulgaris reynaudii*
589 (Cephalopoda, Mollusca) off South Africa: Fighting, guarding, sneaking, mating and egg laying behavior.
590 Bull Mar Sci 71(1): 331-345

591 Hastie LC, Nyegaard M, Collins MA, Moreno A, Pereira JMF, Piatkowski U, Pierce GJ (2009) Reproductive
592 biology of the loliginid squid, *Alloteuthis subulata* in the north-east Atlantic and adjacent waters. Aquat
593 Living Resour 22(1): 35-44. https://doi.org/10.1051/alr/2009002

594 Hatfield E (1991) Post-recruit growth of the Patagonian squid *Loligo gahi* (D'Orbigny). B Mar Sci 49(1-2): 349-
595 361

596 Hatfield EMC (2000) Do some like it hot? Temperature as a possible determinant of variability in the growth of
597 the Patagonian squid, *Loligo gahi* (Cephalopoda: Loliginidae). Fish Res 47(1): 27-40.
598 https://doi.org/10.1016/s0165-7836(99)00127-7

599 Hatfield EMC and Murray AWA (1999) Objective assessment of maturity in the Patagonian squid *Loligo gahi*
600 (Cephalopoda: Loliginidae) from Falkland Islands waters. ICES J Mar Sci 56(5): 746-756.
601 https://doi.org/10.1006/jmsc.1999.0514

602 Hirst AG (2012) Intraspecific scaling of mass to length in pelagic animals: Ontogenetic shape change and its
603 implications. Limnol Oceanogr 57(5): 1579-1590. https://doi.org/10.4319/lo.2012.57.5.1579

Hoar JA, Sim E, Webber DM, O'Dor RK (1994) The role of fins in the competition between squid and fish. Cambridge University Press. https://doi.org/10.1017/cbo9780511983641.004

Jackson GD, Steer BM, Wotherspoon S, Hobday AJ (2003) Variation in age, growth and maturity in the Australian arrow squid *Nototodarus gouldi* over time and space what is the pattern? Mar Ecol Prog Ser 264: 57-71. https://doi.org/10.3354/meps264057

Jiang L, Kang L, Wu C, Chen M, Lü Z (2018) A comprehensive description and evolutionary analysis of 9 Loliginidae mitochondrial genomes. Hydrobiologia 808(1): 115-124. https://doi.org/10.1007/s10750-017-3377-y

Laptikhovsky V (2008) New data on spawning and bathymetric distribution of the Patagonian squid, *Loligo gahi*. Mar Biodivers Rec, 1. https://doi.org/10.1017/s175526720700560x

Lipinski M (1979) Universal maturity scale for the commercially-important squids (Cephalopoda:Teuthoidea). The results of maturity classification of the *Illex illecebrosus* (LeSueur, 1821) population for the years 1973–1977. ICNAF Res Doc, pp 1-39

Lombarte A, Rufino MM, Sánchez P (2006) Statolith identification of Mediterranean Octopodidae, Sepiidae, Loliginidae, Ommastrephidae and Enoploteuthidae based on warp analyses. J Mar Biol Assoc U K 86(4): 767-771. https://doi.org/10.1017/s0025315406013683

Mangold K (1987) Reproduction. In: Boyle PR (ed) Cephalopod life cycles. Vol. II. Academic Press, London, pp 157–200. https://doi.org/10.1017/s0025315400050190

Mesnil B (1977) Growth and life cycle of squid, *Loligo pealei* and *Illex illecebrosus*, from the Northwest Atlantic. ICNAF Selected Papers, 2, pp.55-69.

Moreno A, Azevedo M, Pereira J, Pierce GJ (2007) Growth strategies in the squid *Loligo vulgaris* from Portuguese waters. Mar Biol Res, 3(1): 49-59.

Moreno A, Pereira J, Cunha M (2005) Environmental influences on age and size at maturity of *Loligo vulgaris.* Aquat Living Resour 18(4): 377-384. https://doi.org/10.1051/alr:2005023

628 Neige P (2003) Spatial patterns of disparity and diversity of the recent cuttlefishes (Cephalopoda) across the old
629 world. J Biogeogr 30(8): 1125-1137. https://doi.org/10.1046/j.1365-2699.2003.00918.x

630 Neige P, Boletzky SV (1997) Morphometrics of the shell of three Sepia species (Mollusca: Cephalopoda): intra-
631 and interspecific variation. Zool Beitr 38: 137-156

632 O'Dor R (1998) Can understanding squid life-history strategies and recruitment improve management? S Afr J
633 Marine Sci 20(1): 193-206. https://doi.org/10.2989/025776198784126188

634 Olyott LJH, Sauer WHH, Booth AJ (2006) Spatio-temporal patterns in maturation of the chokka squid (*Loligo*
635 *vulgaris reynaudii*) off the coast of South Africa. ICES J Mar Sci 63(9): 1649-1664.
636 https://doi.org/10.1016/j.icesjms.2006.06.011

637 Patterson K (1988) Life history of Patagonian squid *Loligo gahi* and growth parameter estimates using least-
638 squares fits to linear and Von Bertalanffy models. Mar Ecol Prog Ser: 65-74.
639 https://doi.org/10.3354/meps047065

640 Pecl GT, Jackson GD (2008) The potential impacts of climate change on inshore squid: biology, ecology and
641 fisheries. Rev Fish Biol Fisher 18(4): 373-385. https://doi.org/10.1007/s11160-007-9077-3

642 Pierce GJ, Guerra A (1994) Stock assessment methods used for cephalopod fisheries. Fish Res 21(1-2): 255-
643 285. https://doi.org/10.1016/0165-7836(94)90108-2

644 Pierce GJ, Sauer W, Allcock AL, Smith JM, Wangvoralak S, Jereb P, Hastie LC, Lefkaditou E (2013) *Loligo*
645 *forbesii*, Veined Squid. In: Rosa R, O'Dor R, Pierce GJ (ed) Advances in squid biology, ecology and
646 fisheries. Part I-myopsid squids. Nova Science Publishers Inc, pp 73

647 Raya CP, Balguerías E, Fernández-Núñez MM, Pierce GJ (1999) On reproduction and age of the squid *Loligo*
648 *vulgaris* from the Saharan bank (north-west African coast). J Mar Biol Ass U K 79(1): 111-120.
649 https://doi.org/10.1017/s002531549700012x

650 Rocha F, Guerra A (1999) Age and growth of two sympatric squid *Loligo vulgaris* and *Loligo forbesi*, in
651 Galician waters (north-west Spain). J Mar Biol Ass UK 79(4): 697-707.
652 https://doi.org/10.1017/s002531549800085x

Rodríguez-Mendoza R, Muñoz M, Saborido-Rey F (2011). Ontogenetic allometry of the bluemouth, *Helicolenus dactylopterus dactylopterus* (Teleostei: Scorpaenidae), in the Northeast Atlantic and Mediterranean based on geometric morphometrics. Hydrobiologia 670(1): 5–22. https://doi.org/10.1007/s10750-011-0675-7

Rohlf FJ (2015) The tps series of software. Hystrix 26(1). https://doi.org/10.4404/hystrix-26.1-11264

Sauer W (1995) South Africa's Tsitsikamma national park as a protected breeding area for the commercially exploited chokka squid *Loligo vulgaris reynaudii*. S Afr J Marine Sci 16(1): 365-371. https://doi.org/10.2989/025776195784156575

Schroeder R, Schwarz R, Crespi-Abril AC, Alvarez Perez JA (2017) Analysis of shape variability and life history strategies of *Illex argentinus* in the northern extreme of species distribution as a tool to differentiate spawning groups. J Nat Hist 51(43-44): 2585-2605. https://doi.org/10.1080/00222933.2017.1374484

Shashar N, Hanlon RT (2013) Spawning behavior dynamics at communal egg beds in the squid *Doryteuthis (Loligo) pealeii*. J Exp Mar Biol Ecol 447: 65-74. https://doi.org/10.1016/j.jembe.2013.02.011

Team RC (2016) R: A language and environment for statistical computing. R Foundation for Statistical Computing 2015. Vienna, Austria

van der Vyver J, Sauer W, McKeown N, Yemane D, Shaw P, Lipinski M (2016) Phenotypic divergence despite high gene flow in chokka squid *Loligo reynaudii* (Cephalopoda: Loliginidae): Implications for fishery management. J Mar Biol Assoc U K 96(7): 1507-1525. https://doi.org/10.1017/s0025315415001794

Vega MA, Rocha FJ, Osorio C (2001) Morfometría comparada de los estatolitos del calamar *Loligo gahi* d'Orbigny, 1835 (Cephalopoda: Loliginidae) del norte de Perú e islas Falkland. Investigaciones marinas 29(1): 3-9. https://doi.org/10.4067/s0717-71782001000100001

Vega MA, Rocha FJ, Guerra A, Osorio C (2002) Morphological differences between the Patagonian squid *Loligo gahi* populations from the Pacific and Atlantic oceans. Bull Mar Sci 71(2): 903-912

676 Vila Y, Silva L, Torres MA, Sobrino I (2010) Fishery, distribution pattern and biological aspects of the common
677 European squid *Loligo vulgaris* in the Gulf of Cadiz. Fish Res 106(2): 222-228.
678 https://doi.org/10.1016/j.fishres.2010.06.007

679 Wada T, Takegaki T, Mori T, Natsukari Y (2005) Alternative male mating behaviors dependent on relative body
680 size in captive oval squid *Sepioteuthis lessoniana* (Cephalopoda, Loliginidae). Zool Sci 22(6): 645-651.
681 https://doi.org/10.2108/zsj.22.645

682 Wagenmakers EJ, Farrell S (2004) AIC model selection using Akaike weights. Psychon Bull Rev 11: 192-196.
683 https://doi.org/10.3758/bf03206482

684 Winter A, Arkhipkin A (2015). Environmental impacts on recruitment migrations of Patagonian longfin squid
685 (*Doryteuthis gahi*) in the Falkland Islands with reference to stock assessment. Fish Res 172: 85-95.
686 https://doi.org/10.1016/j.fishres.2015.07.007

687 Wood SN (2011) Fast stable restricted maximum likelihood and marginal likelihood estimation of
688 semiparametric generalized linear models. J R Stat Soc Series B 73(1): 3-36.
689 https://doi.org/10.1111/j.1467-9868.2010.00749.x

690 Zelditch ML, Lundrigan BL, David Sheets H, Garland Jr T (2003) Do precocial mammals develop at a faster
691 rate? A comparison of rates of skull development in *Sigmodon fulviventer* and *Mus musculus domesticus*. J
692 Evol Biol 16(4):708-720. https://doi.org/10.1046/j.1420-9101.2003.00568.x

693 Zuur AF, Ieno EN, Elphick CS (2010) A protocol for data exploration to avoid common statistical problems.
694 Methods Ecol Evol 1(1): 3-14. https://doi.org/10.1111/j.2041-210x.2009.00001.x

Figures

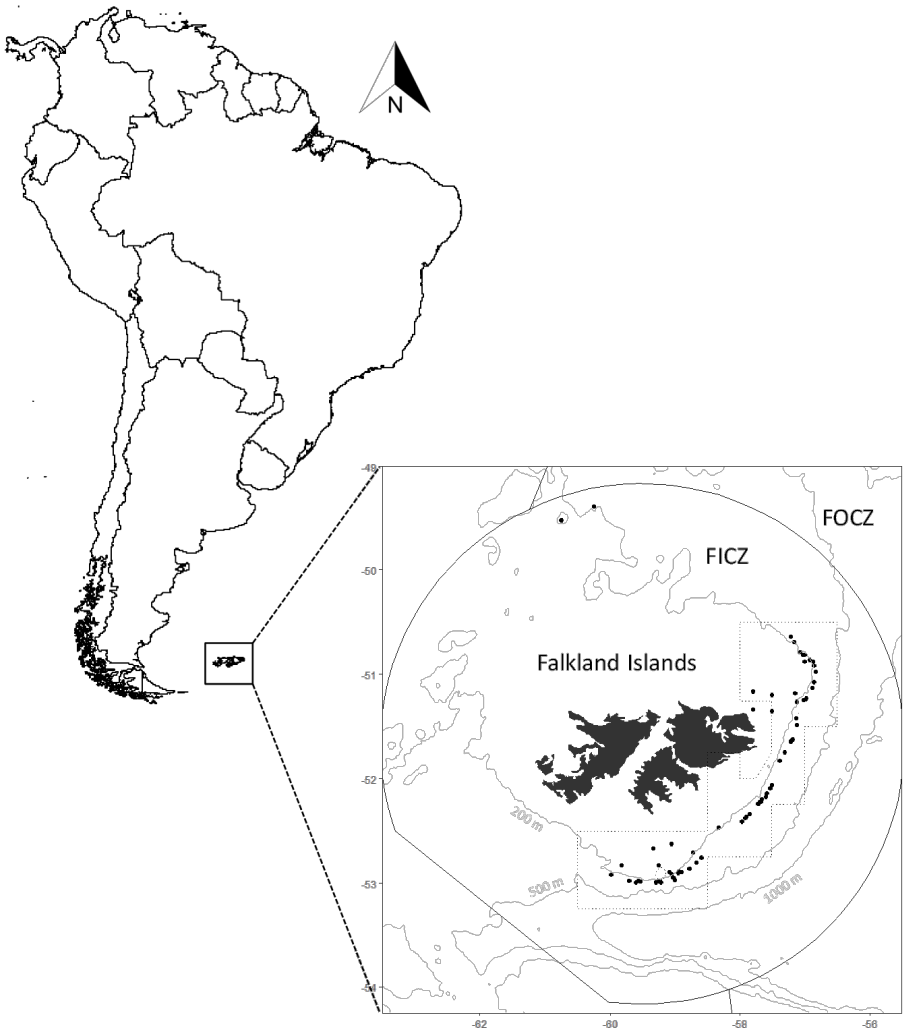


Fig. 1 Map showing the Falkland Islands Inner Conservation Zone (FICZ – the sample area), outer conservation zone (FOCZ), Loligo box (dotted line) and the position of the Falkland Islands relative to mainland South America with points showing the sample sites

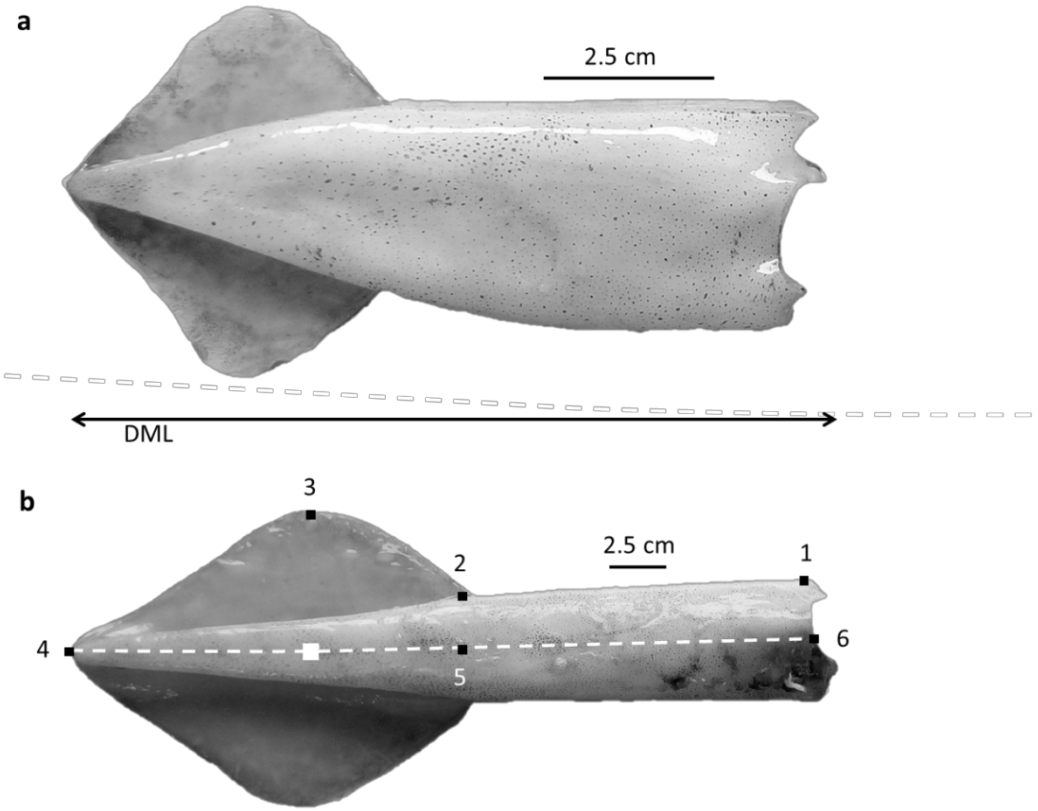


Fig. 2 a - squid of modal population length with arrows dorsal mantle length (DML), scale bars included **b -** large 'super-bull' males labelled with landmark configurations adapted from Crespi-Abril et al. (2010). Dotted line represents sagittal plane. An additional landmark was included to investigate un-bending specimen, indicated by the white square

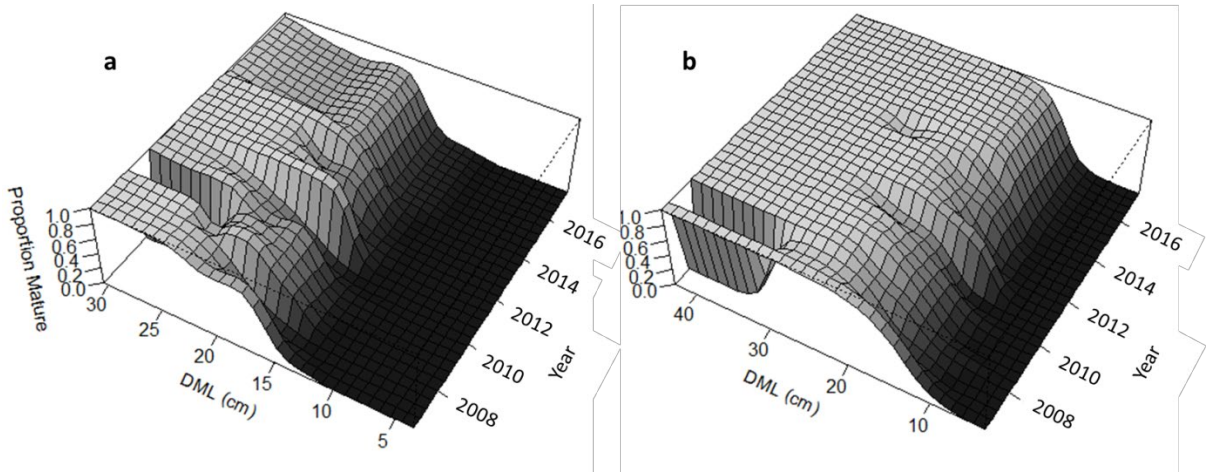


Fig. 3 Partial effects of the binomial GAM MN3 (Table 2) on maturity for dorsal mantle length (DML) with separate smoothing splines fitted for each year for **a –** females and **b –** males

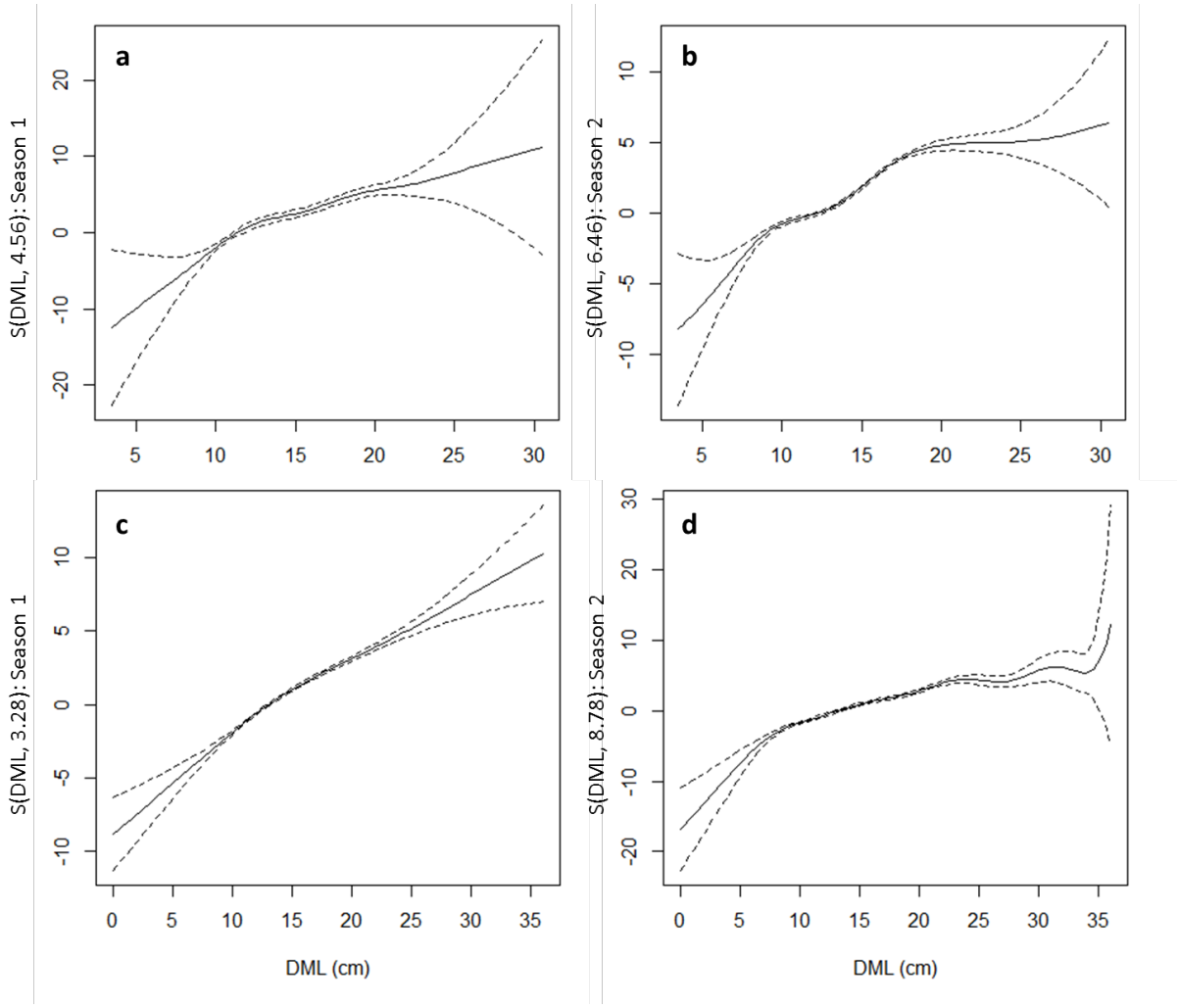


Fig. 4 Partial effects from binomial GAM MN 7 (Table 2) of dorsal mantle length (DML) with separate smoothers for **a** – females in commercial fishing season 1 **b** – females in season 2 **c** – males in season 1 and **d** – males in season 2, with 95% confidence limits (dotted lines)

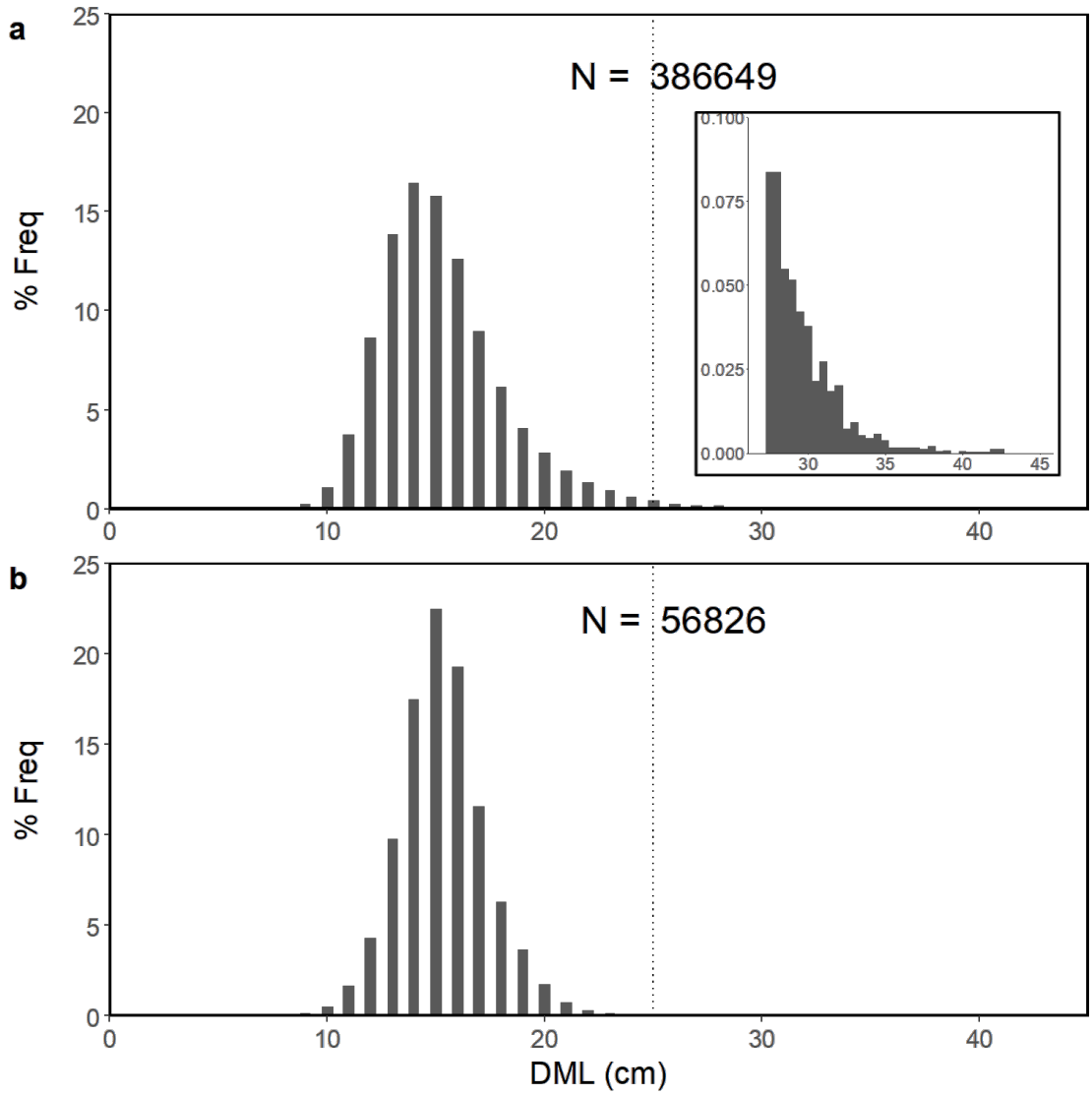


Fig. 5 Length-frequency graphs of **a** – male (with zoomed insert showing the percentage frequency (% Freq) for individuals exceeding 25 cm) and **b** – female *Doryteuthis gahi* in Falkland Islands waters. Sample size (N) included. Dotted line indicates 25 cm dorsal mantle length (DML)

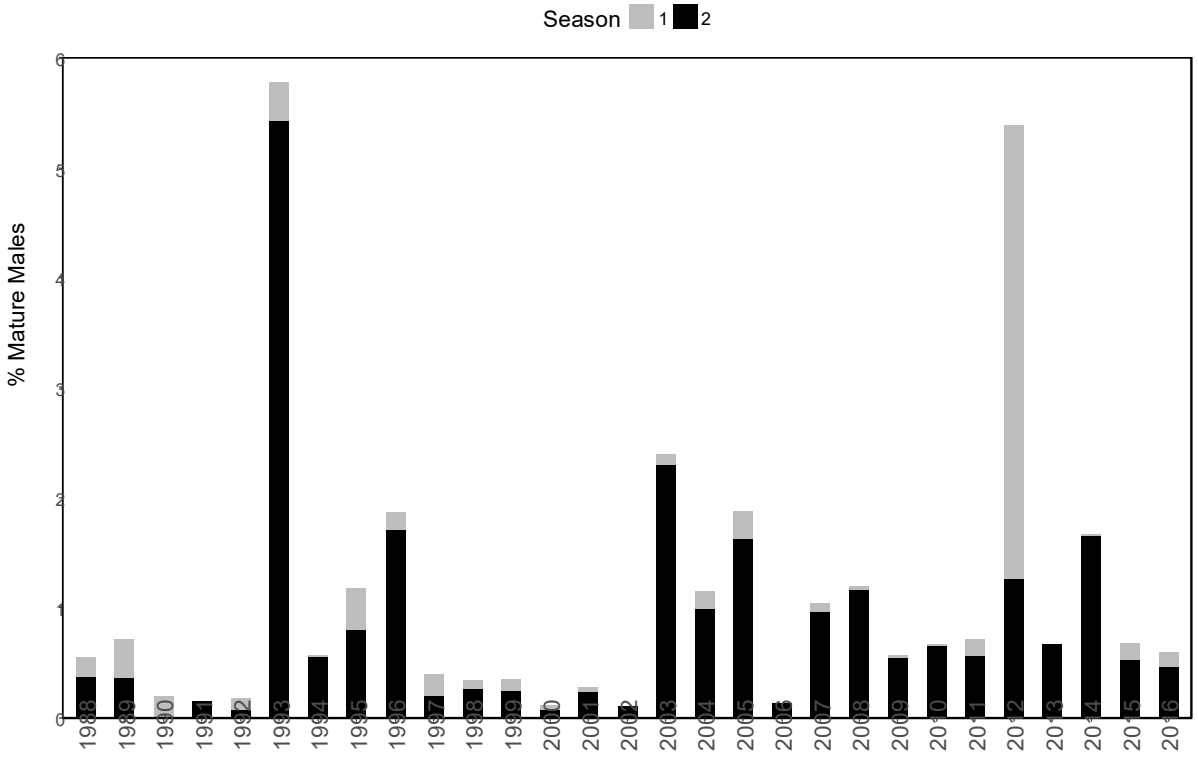


Fig. 6 Percentage of mature males (stage 5 on Lipinski's scale, 1979) exceeding 25 cm dorsal mantle length (DML) in each year, separated into fishing seasons

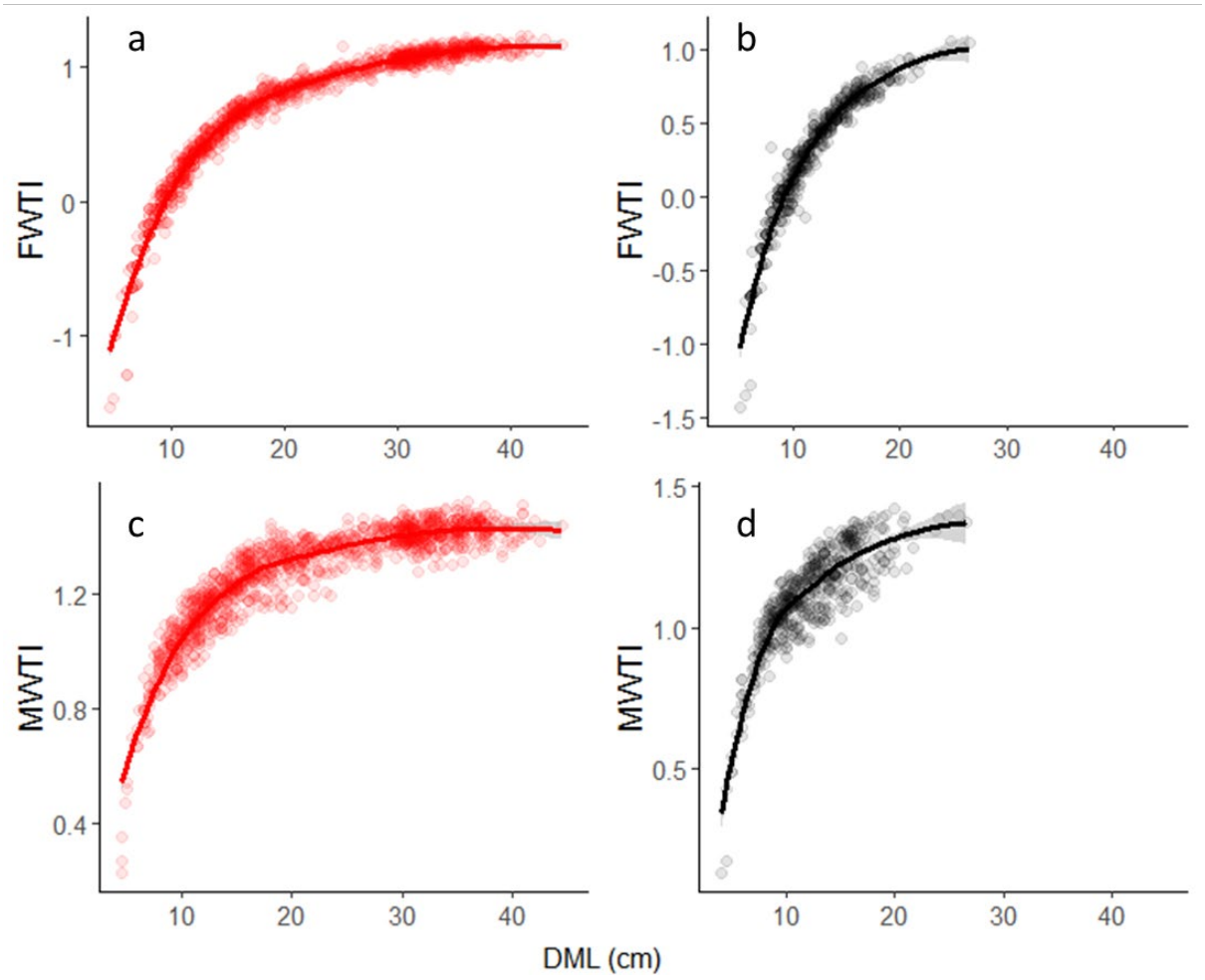


Fig. 7 Indices of fin weight index (FWTI) for **a** – males and **b** - females and mantle weight index (MWTI) for **c** – males and **d** - females plotted against dorsal mantle length (DML). Loess curves with error bars (grey shaded region) fitted to aid in visual interpretation

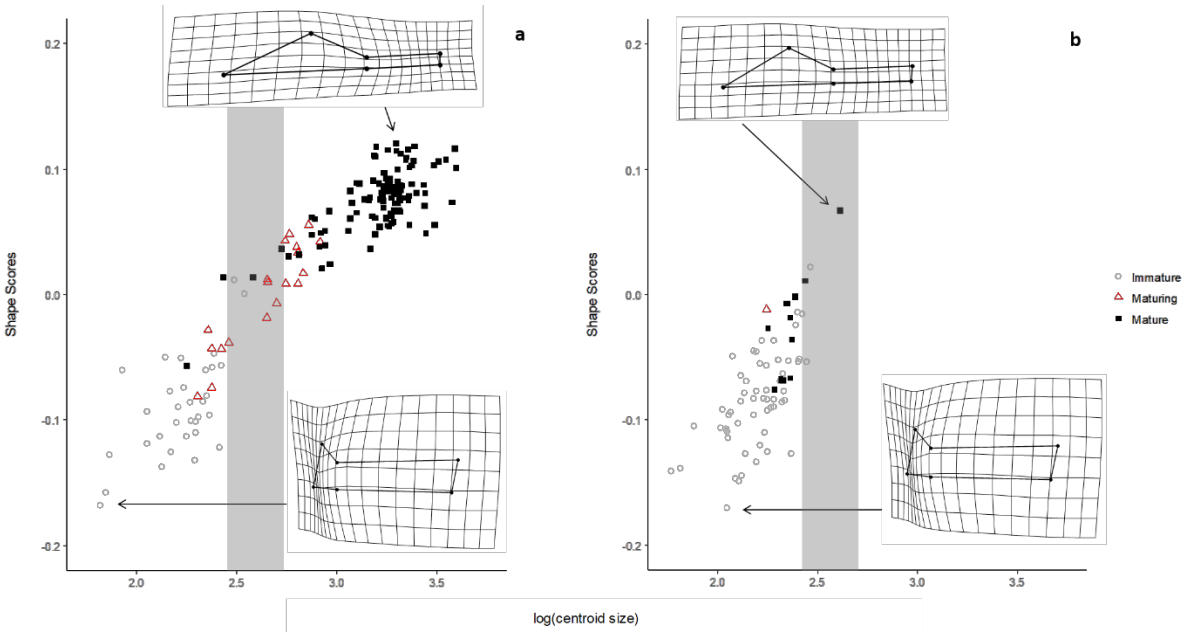


Fig. 8 The corresponding shape scores for *Doryteuthis gahi* included in the study as a function of log(centroid size) for **a** – males and **b** - females, with symbols indicating whether an individual is immature (stages 1-2), maturing (stages 3-4) or mature (stage 5) and thin-plate spline (TPS) deformation grids included for data extremes, magnified x 2. Grey region shows the approximate average adult size range for the population, assuming a linear relationship between CS and dorsal mantle length.

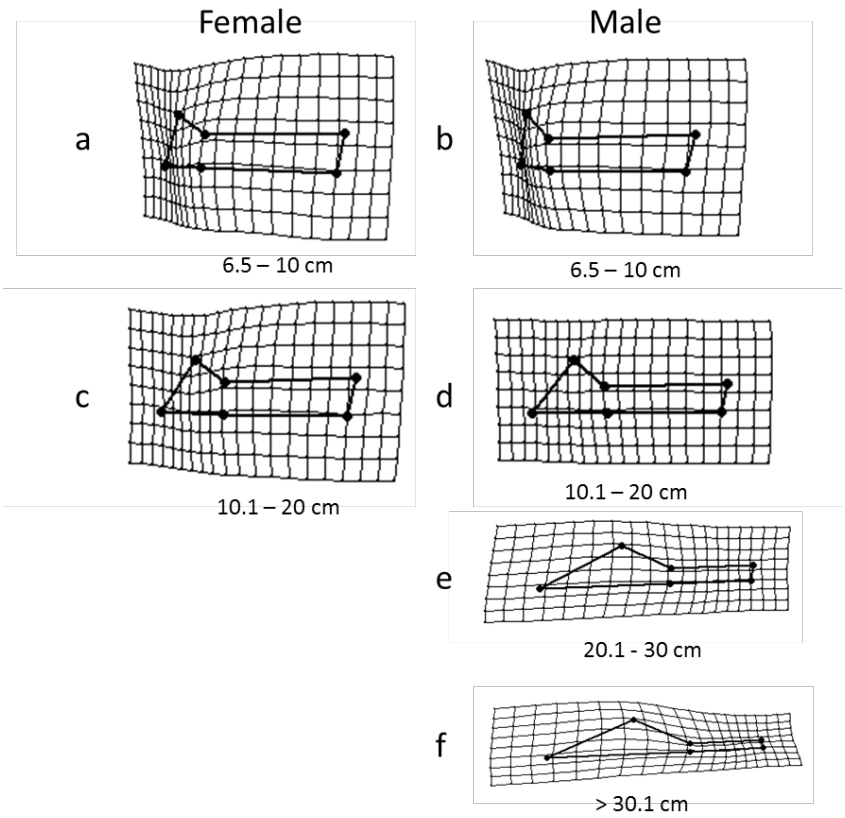


Fig. 9 Visualisation of shape changes associated with sex and size in *Doryteuthis gahi*. Deformation grids show the change from the consensus shape to the mean shape of each size group separated into females (**a/c**) and males (**b/d/e/f**), magnified x 2 to improve visualisation.

Tables

Table 1 Summary statistics of the geometric morphometric dataset for *Doryteuthis gahi.*

Sex	Year	Maturity	N	DML Range (cm)	Mean DML (cm) ± SE
F	2016	immature	58	6.5-14.1	10.3 ± 0.2
	2016	mature	1	16.8	NA
	2017	maturing	2	11.3-24.8	18.1 ± 6.8
	2017	mature	10	11.1-13.2	12 ± 0.2
M	2014	mature	5	30.3-42.5	36.2 ± 2.7
	2015	mature	7	24.8-37.3	30.3 ± 1.67
	2016	immature	32	6.5-15.8	10.5 ± 0.36
	2016	maturing	19	11-21.6	16.7 ± 0.76
	2016	mature	81	11.7-36.1	28.6 ± 0.57
	2017	mature	15	24.2-41.5	34.6 ± 1.19

Table 2 Binomial generalised additive models applied to *Doryteuthis gahi* maturity data, where m is the maturity (0 = immature, 1 = mature) and εi ~ N(0, σ^2). The notation *f*(DML):year means that one smoother is fitted for each year, and year is also fitted as a mean term. Degrees of freedom (df), percentage deviance explained (%DE), Akaike's information criterion (AIC), changes in AICs (ΔAIC) and Akaike weights (AIC wt.) reported for each sex.

		Males (n = 44,439)					Females (n = 46,269)				
MN	Expression	df	%DE	AIC	ΔAIC	AIC wt.	df	%DE	AIC	ΔAIC	AIC wt.
1	$m \sim f(DML) + \varepsilon_i$	5	29.3	39480	8575	<0.001	7	31.5	17597	5952	<0.001
2	$m \sim f(DML) + year + \varepsilon_i$	15	33.3	37269	6364	<0.001	17	33.5	17117	5471	<0.001
3	$m \sim f(DML):year + \varepsilon_i$	56	34.3	36803	5898.6	<0.001	59	34.9	16820	5174	<0.001
4	$m \sim f(DML) + season + \varepsilon_i$	10	37.3	34987	4082.1	<0.001	8	36.2	16389	4743	<0.001
5	$m \sim f(DML):season + \varepsilon_i$	14	37.4	34968	4063.2	<0.001	13	36.5	16334	4688	<0.001
6	$m \sim f(DML):season + year + \varepsilon_i$	24	40.6	33222	2317.2	<0.001	23	38	15971	4326	<0.001
7	$m \sim f(DML):season + f(lon,lat) + year + \varepsilon_i$	53	44.8	30904	0	0.999	50	55	11646	0	0.999

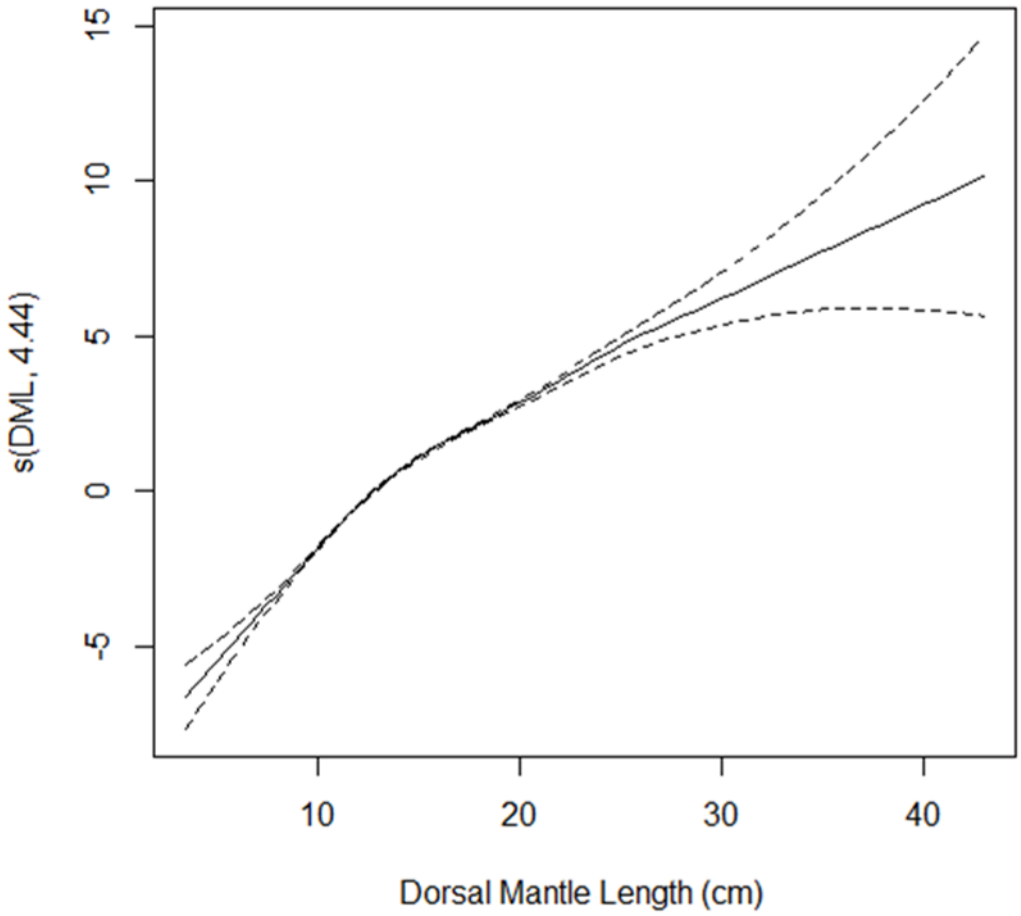
Table 3 Nonparametric multivariate analysis of variance (MANOVA) statistics based on a randomised residual permutation procedure (10,000 iterations) on geometric shape coordinates. Size is log(centroid size), year is year of collection

Co-variables	df	SS	MS	R^2	F	Z	p
Size	1	1.363	1.363	0.726	657.53	6.56	<0.001
Sex	1	0.004	0.004	0.002	1.90	2.99	<0.001
Year	3	0.011	0.004	0.006	1.82	4.67	<0.001
Size:sex	1	0.019	0.019	0.010	9.38	5.19	<0.001
Size:year	3	0.025	0.008	0.013	3.97	6.48	<0.001
Sex:year	1	0.0001	0.0001	0.0001	0.08	-1.15	0.87
Size:sex:year	1	0.003	0.003	0.002	1.42	2.78	<0.001
Residuals	**218**	**1.877**	**0.002**				

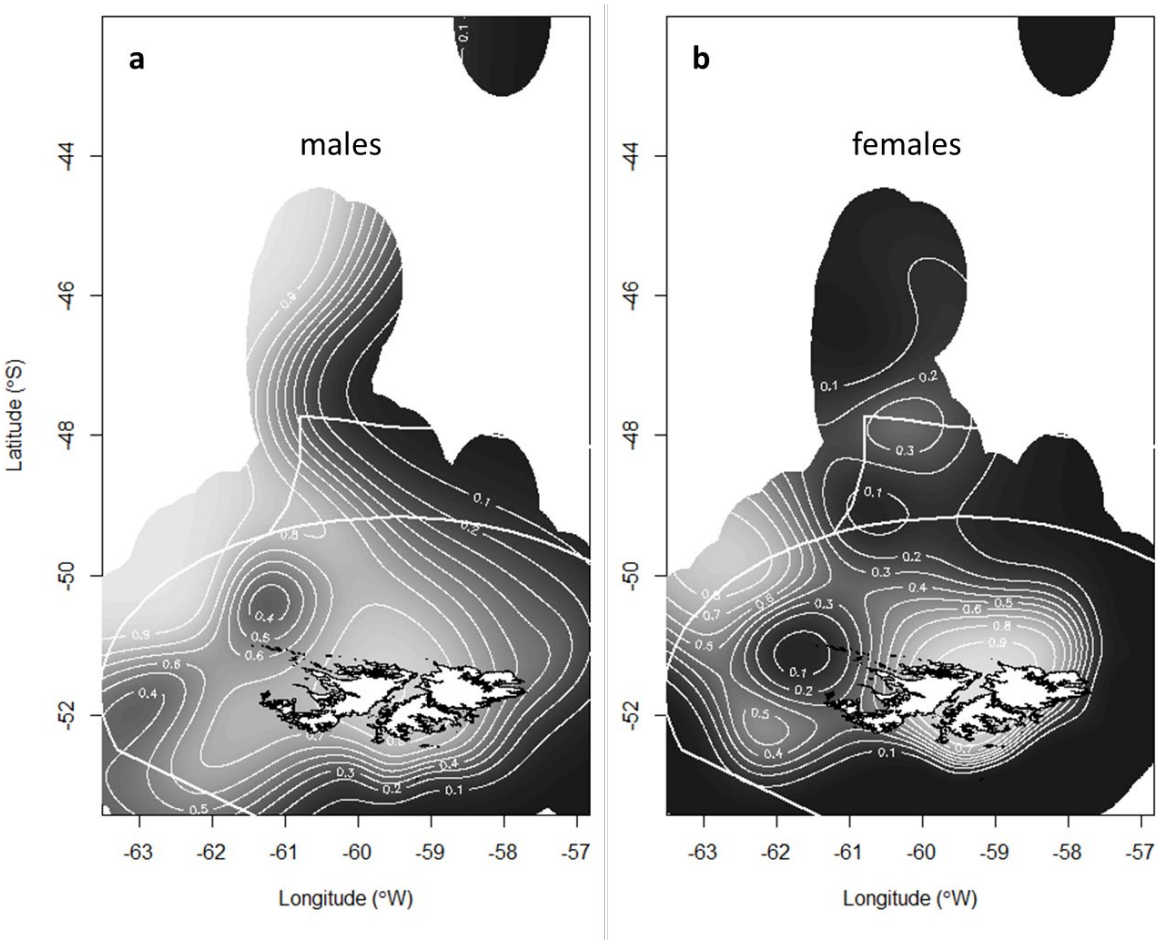
Table 4 Pairwise comparisons of regression vector angle for size groups. * indicates angles are significantly different at the 5% level

	6.5-10	10.1-20	20.1-30	30.1+
6.5-10	0			
10.1-20	25.5	0		
20.1-30	60.5*	41.3*	0	
30.1+	69.9*	79.0*	76.7*	0

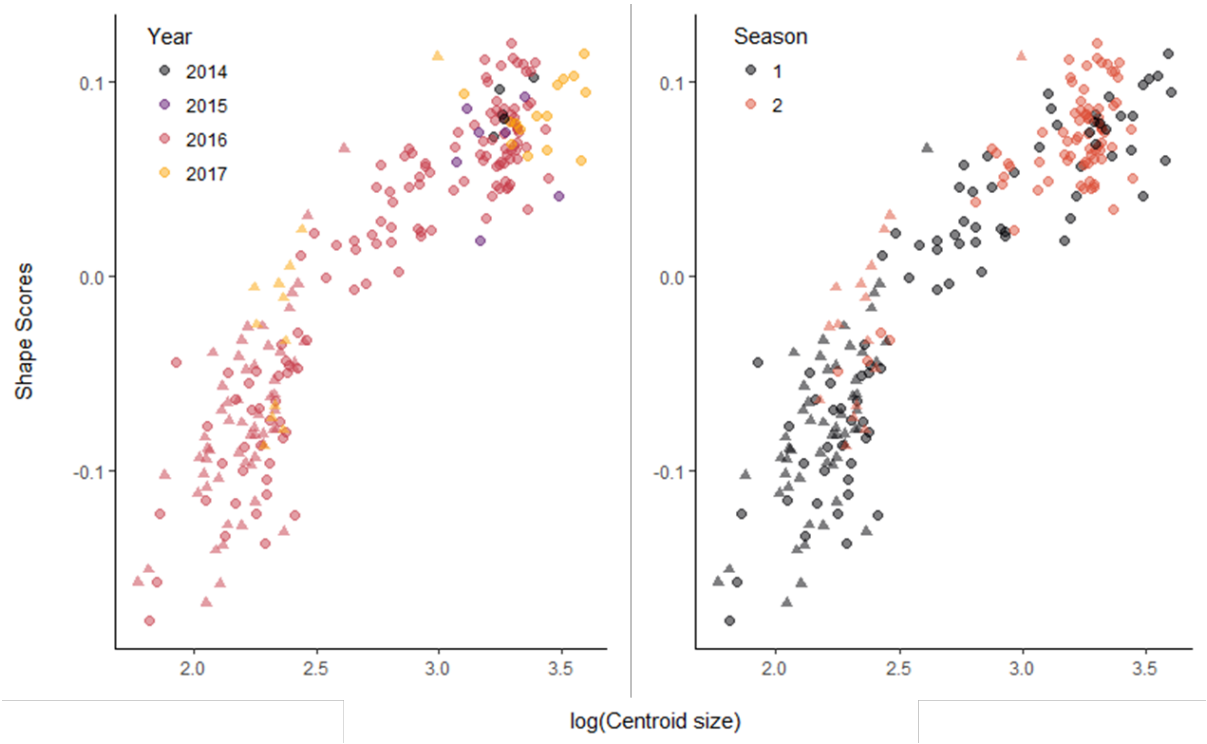
Appendix



Appendix 1 Estimated smoother from MN1 for the effect of dorsal mantle length (DML) in male *Doryteuthis gahi* with dotted lines indicating 95% confidence intervals. Expected degrees of freedom given in smoothing term



Appendix 2 Contour plots for the partial effects from the GAM MN7 (Table 2) for **a –** males and **b -** females of longitude/latitude. Higher values indicate mature individuals. Position of the Falkland Islands included; thick white line indicates the Falkland Islands conservation zones (FICZ and FOCZ)



Appendix 3 The corresponding shape scores for *Doryteuthis gahi* included in the study as a function of log(centroid size) coloured by year and season, with symbols indicating whether an individual is male (circles) or female (triangles)

Appendix 4 Nonparametric multivariate analysis of variance (MANOVA) statistics based on a randomised residual permutation procedure (10,000 iterations) on geometric shape coordinates with non-random samples removed. Size is log(centroid size)

Co-variables	**df**	**SS**	**MS**	**R^2**	**F**	**Z**	**p**
Size	1	0.431	0.431	0.551	177.22	5.94	<0.001
Sex	1	0.009	0.008	0.01	3.22	3.07	<0.001
Size x sex	1	0.007	0.007	0.009	3.05	3.04	<0.001
Residuals	**138**	**0.336**	**0.002**				